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single base. After comparing the probe intensities, an unknown base is identified (typically as A, C, G, or T) according to the results of the comparison. In one embodiment, a ratio is calculated between the highest probe intensity and the next highest probe intensity. If the ratio is greater than a predetermined ratio value (e.g., 1.2), the unknown base is identified according to nucleic acid probe that produced the highest probe intensity.

The Cited Art Distinguished

Claims 1, 3-20 and 45-59 were rejected under 35 U.S.C. § 103 as being unpatentable over Fodor in view of Weiss and Stockham. Fodor describes, among other things, pioneering techniques for sequencing by hybridization. However, the Examiner cited Weiss and Stockham for disclosing the base calling (identifying) methods of the present invention. For the following reasons, these references do not disclose or suggest the present invention as claimed.

Weiss and Stockham are related to nucleic acid sequencing which utilizes nucleic acid ladders which may be formed by well known techniques such as the Sanger dideoxy method or the Maxam and Gilbert method. More specifically, Weiss describes utilizing an enzyme on identical probes that hybridize with tags in the fragments of the nucleic acid ladder. The enzymes convert a fluorogenic substrate (e.g., BBTP) into a fluorescent product in order to enhance the pattern of hybridization (see, e.g., Fig. 1C).

Stockham, more specifically, describes methods of sharpening signal peaks from electrophoretic migration patterns of nucleic acid ladders. Each fragment of the nucleic acid ladder is labeled with a radioactive label which is utilized to identify the position of the fragment on the gel following electrophoresis. As analyzing the migration patterns is time consuming and often error prone, Stockham describes equations and formulas for increasing the accuracy of this process (e.g., sharpening signal peaks).

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Weiss and Stockham do not disclose or suggest inputting probe intensities to identify an unknown base where the probe intensities indicate the extent of hybridization of probes differing by a single base and the sample nucleic acid sequence. Claim 60 recites the following:

inputting a plurality of probe intensities for a plurality of nucleic acid probes, each probe intensity indicating an extent of hybridization of a nucleic acid probe with at least one nucleic acid sequence including said sample sequence, and each nucleic acid probe differing from each other by a single base;

(emphasis supplied). Neither Weiss nor Stockham discloses these limitations.

Initially, Weiss uses a single probe which will hybridize to a tag on the nucleic acid ladder fragments. As such, all of the "probes" in Weiss are identical. Furthermore, the probes in Weiss do not indicate the extent of hybridization but instead are utilized to generate a fluorescent signal which indicates the location of a fragment on the substrate. Accordingly, it is the location of the fragments that is utilized to sequence a nucleic acid.

Stockham does not utilize probes at all. Instead, Stockham recites that the fragments of the nucleic acid ladder are radioactively labeled. The radioactive signal resulting indicates the position of the fragments on the gel in a way which is similar to Weiss. Accordingly, Stockham also utilizes the location of the fragments to sequence a nucleic acid.

In stark contrast, the present invention compares probe intensities that indicate the extent of hybridization of probes differing by a single base and the sample nucleic acid sequence. Claim 60 recites the following:

said computer system comparing said plurality of probe intensities; and
identifying said unknown base according to results of said comparing step.

In the Office Action, the Examiner stated that it would have been prima facie obvious to one of ordinary skill in the art to use the computer algorithms of Weiss and Stockham to interpret that data from the sequencing by hybridization described by Fodor. More specifically, the Examiner stated that one could "call" a

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site based on the intensity of a signal produced by a probe at that site and thus assign an identity to that site. Applicants disagree.

Weiss and Stockham relate to vastly different technologies than the pioneering advances of Fodor. Weiss and Stockham are directed to identifying the location of a fragment of a nucleic acid ladder. In the present invention, the locations of the hybridized probes are known and, as such, the computer algorithms of Weiss and Stockham would indeed seem to teach away from the present invention which is directed to calling an unknown base according to probe intensities from nucleic acid probes that differ by a single base.

As Weiss and Stockham do not disclose or suggest all the limitations of claim 60, the claim is patentably distinct over the references. All the other pending claims contain similar limitations. Therefore, Applicants request that all the pending claims be passed to issue.

Other Claims

Independent claims 81, 88 and 99 recite specific methods of identifying unknown bases. Details on specific embodiments of these methods may be found in the specification under the headings "Intensity Ratio Method," "Reference Method" and "Statistical Method." These claims recite methods that are patentable for at least the same reasons as above. Additionally, these claims include further limitations that make them further patentably distinct.

Claim 81 recites that a ratio of a higher probe intensity and a lower probe intensity is calculated. Then, the unknown base is identified according to the probe that had the higher probe intensity if the ratio is greater than a predetermined ratio value. Weiss and Stockham simply do not disclose or suggest this method. Accordingly, claims 81-87 are patentably distinct.

Claim 88 recites that probe intensities from a first set of probe intensities from probes hybridizing with a reference nucleic acid sequence, and a second set of probe intensities from

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probes hybridizing with a sample nucleic acid sequence are compared. Based on this comparison, the unknown base is identified. Weiss and Stockham do not disclose or suggest this method. Accordingly, claims 88-98 are patentably distinct.

Claim 99 recites that a probe intensity of a nucleic acid probe hybridizing with a sample sequence is compared to statistics from nucleic acid probes hybridizing with a reference sequence. Based on this comparison, the unknown base is identified. Weiss and Stockham do not disclose or suggest this method. Accordingly, claims 99-105 are patentably distinct.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (415) 326-2400.

Respectfully submitted,

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